

# Blm3 is part of nascent proteasomes and is involved in a late stage of nuclear proteasome assembly

Marion Fehlker\*, Petra Wendler\*, Andrea Lehmann\* & Cordula Enenkel<sup>†</sup>

Institut für Biochemie, Humboldt Universität zu Berlin, Berlin, Germany

Proteasomes are multisubunit proteases that are responsible for regulated proteolysis. The degradation of the proteasomal maturation factor, named Ump1 in yeast, completes the autocatalytic processing of inactive precursor complexes into the proteolytically active core particle (CP) of the proteasome. We have identified Blm3, a conserved nuclear protein, as a new component of Ump1-associated precursor complexes. A lack of Blm3 resulted in an increased rate of precursor processing and an accelerated turnover of Ump1, which suggests that Blm3 prevents premature activation of proteasomal CPs. On the basis of biochemical fractionation experiments combined with *in vivo* localization studies, we propose that Blm3 joins nascent CPs inside the nucleus to coordinate late stages of proteasome assembly in yeast.

EMBO reports 4, 959–963 (2003)

doi:10.1038/sj.embor.embor938

## INTRODUCTION

Proteasomes, the main proteases in the nucleoplasm and cytoplasm of eukaryotic cells, are involved in a variety of biological processes. Two multisubunit complexes, the proteolytically active core particle (CP) and the regulatory particle (RP), constitute the proteasome. The RP recognizes protein substrates, gates the CP to translocate substrates into its catalytic cavity and confers ATP dependence on protein degradation (Glickman & Maytal, 2002).

The CP consists of two rings of seven  $\beta$ -subunits, which contains the active sites, flanked by two rings of seven non-catalytic  $\alpha$ -subunits. The assembly of the CP is a multistep reaction, in which rings of  $\alpha$ -subunits associated with pro- $\beta$  subunits form early assembly intermediates (Frentzel *et al.*, 1994). In yeast, early assembly intermediates are known as 15S precursor complexes or half-assembled core particles (h-CPs; Chen & Hochstrasser, 1996; Ramos *et al.*, 1998). According to current models, the dimerization of two h-CPs into an as yet uncharacterized, short-lived late assembly intermediate has to take place before the release of active sites is triggered by the autocatalytic processing of distinct pro- $\beta$  subunits (Chen & Hochstrasser, 1996). These late assembly intermediates are known as unstable

processing intermediates, pre-holo-proteasomes or nascent CPs (n-CPs). A conserved maturation factor, originally named Ump1 in yeast, is associated with the h-CP. Trapped inside the n-CP, Ump1 ensures efficient active-site formation and becomes the first substrate of the n-CP (Ramos *et al.*, 1998; Heinemeyer, 2000; Krüger *et al.*, 2001).

In yeast, ~80% of proteasomes are localized in the nuclear periphery, which is the main subcellular location of proteasomal proteolysis (Enenkel *et al.*, 1998; Wilkinson *et al.*, 1998; Russell *et al.*, 1999). Moreover, we found that Ump1-associated precursor complexes that resemble h-CPs are mainly nuclear in yeast. Our data suggested that h-CPs are imported into the nucleus before being assembled into the active CP and that proteasomal maturation takes place at the subcellular site of proteasomal proteolysis (Lehmann *et al.*, 2002).

To gain further insight into the pathway of nuclear CP assembly, we screened Ump1-associated precursor complexes from nuclear extracts for new interacting proteins and discovered Blm3. *In vivo* localization studies verified that Blm3 is a nuclear protein. Using native gel electrophoresis and green fluorescent protein (GFP)-labelling techniques, Ump1-associated precursor complexes were fractionated into h-CPs and n-CPs. Blm3 was absent from the h-CP fraction but part of the n-CP fraction. We found that the kinetics of precursor processing are accelerated in *blm3Δ* null mutants compared with wild-type cells, which indicates that Blm3 delays the conversion of the n-CP into the mature CP. We propose that Blm3 is a new coordinator of nuclear CP maturation in yeast.

## RESULTS AND DISCUSSION

Recent studies from our laboratory carried out in yeast suggested that the nuclear CP originates from Ump1-associated precursor complexes that are localized in the nucleus. To identify new components involved in nuclear CP assembly and maturation, we analysed the protein composition of Ump1-associated precursor complexes from nuclear and endoplasmic reticulum (ER) extracts using IgG affinity-chromatography of protein-A-tagged Ump1 (Lehmann *et al.*, 2002). In such precursor preparations, we consistently co-purified a 240-kDa protein (Fig. 1A). Peptide sequences derived from the 240-kDa protein were searched for in the yeast genome database and were assigned to Blm3, the gene product of *BLM3-YFL006* (see supplementary information online). Blm3 was also found in preparations of proteasomal complexes that were affinity purified using protein-A-tagged versions of  $\alpha$ -subunits (data not shown; Gavin *et al.*, 2002).

The name of the *BLM3* gene originated from a genetic study in yeast. *BLM3* was cloned by complementing the bleomycin

Institut für Biochemie, Humboldt Universität zu Berlin, Universitätsklinikum Charité, Monbijoustrasse 2, D-10117 Berlin, Germany

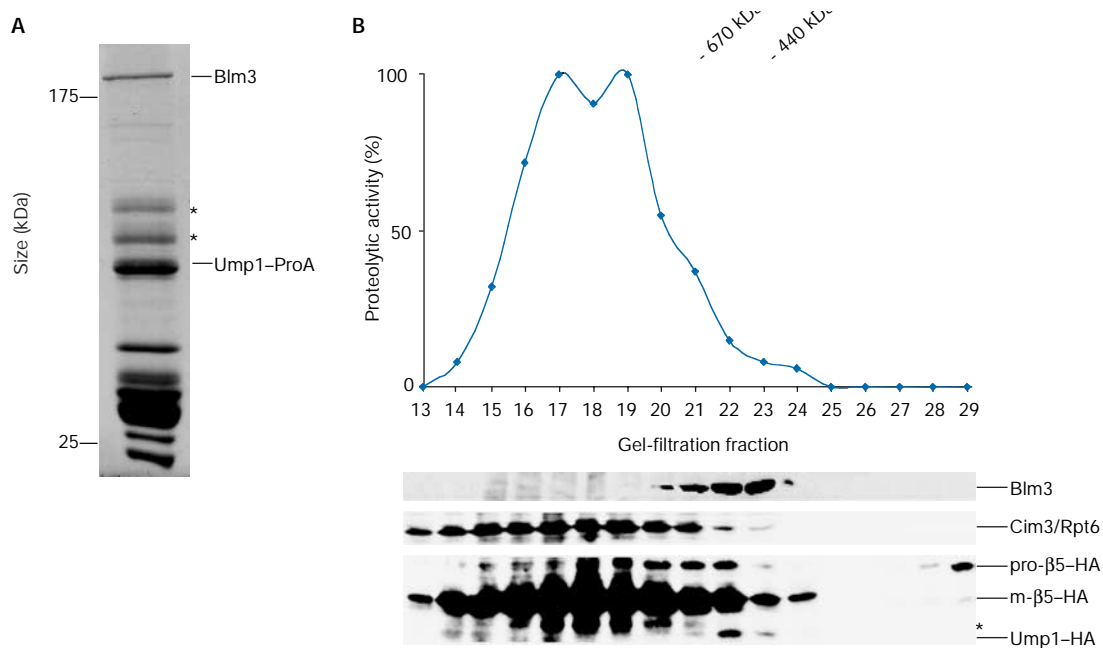
\*These authors contributed equally to this work

<sup>†</sup>Corresponding author. Tel: +30 450 528158; Fax: +30 450 528916;

E-mail: cordula.enenkel@charite.de

Received 3 April 2003; revised 17 July 2003; accepted 8 August 2003

Published online 12 September 2003



**Fig. 1** | Identification of Blm3 as part of Ump1-associated precursor complexes. (A) Coomassie-blue-stained SDS-polyacrylamide gel of Ump1-associated precursor complexes that were isolated by IgG affinity-chromatography from nuclear/endoplasmic reticulum extracts from yeast wild-type cells expressing protein A (ProA)-tagged Ump1 instead of the endogenous protein (Lehmann *et al.*, 2002). Blm3 and Ump1-ProA are indicated. Proteins indicated by asterisks are heat shock proteins. (B) Extracts from wild-type cells expressing haemagglutinin (HA)-tagged  $\beta$ 5 and Ump1 instead of the endogenous proteins (wild-type strain JD133; Ramos *et al.*, 1998) were fractionated on Superose 6 (Amersham). Fractions were assayed for peptide-cleavage activity against Cbz-Leu-Leu-Glu- $\beta$ -naphthylamide (upper panel). Protein samples of each fraction were run on SDS-polyacrylamide gels, blotted and probed for Blm3, the regulatory particle subunit Cim3/Rpt6, the core particle subunit  $\beta$ 5-HA and Ump1-HA (lower panel). Unprocessed and matured  $\beta$ 5 subunits are abbreviated as pro- $\beta$ 5 and m- $\beta$ 5, respectively. A degradation band of m- $\beta$ 5 is indicated by an asterisk. The Superose 6 column was calibrated using the standards thyroglobulin (670 kDa) and ferritin (440 kDa).

hypersensitivity that is conferred by the *blm3-1* mutation, which suggests that Blm3 functions in protecting cells from DNA damage (Evans Febres *et al.*, 2001). A structural homologue of Blm3 exists in mammalian cells, and has 17% identity with Blm3 and is named PA200 (Ustrell *et al.*, 2002).

Before we continued our biochemical studies of the function of Blm3 in CP assembly, we used basic yeast genetics. First, we deleted the *BLM3-YFL006* gene. The resulting *blm3 $\Delta$*  null mutant is viable, but temperature sensitive (see supplementary information online), indicating that Blm3 is required under stress conditions. Second, we overexpressed Blm3 by chromosomal disruption of the *BLM3* promoter using an inducible *GAL1* promoter. The overexpression of Blm3 was found to inhibit cell growth (see supplementary information online).

Further evidence that Blm3 is a precursor-interacting protein was provided by gel-filtration experiments and density-gradient ultracentrifugations. Both fractionation methods showed that Blm3 does not coincide with the peak fractions of active proteasomes. In gel-filtration experiments, Blm3 co-eluted with Ump1-associated precursor complexes (Fig. 1B). In density-gradient ultracentrifugation experiments, Blm3 co-sedimented with fractions that comprised Ump1-associated precursor complexes (see supplementary information online).

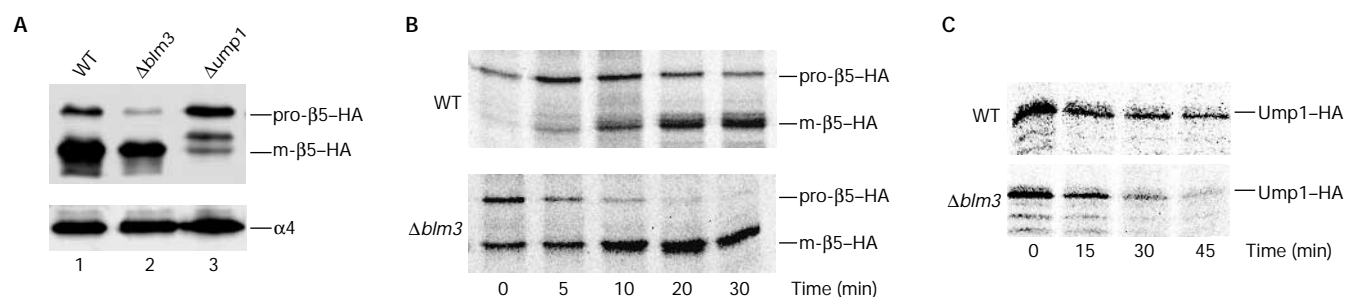
If Blm3 functions in CP assembly and maturation, a deficiency for Blm3 may affect CP maturation, as seen in cells lacking Ump1 (Ramos *et al.*, 1998). The lack of Ump1 causes the stabilization of precursor complexes, which is manifested in increased levels of pro- $\beta$ 5 subunits, the crucial determinants of CP maturation

(Chen & Hochstrasser, 1996; Jäger *et al.*, 1999). To test how a deletion of *BLM3* affects CP maturation, steady-state levels of pro- $\beta$ 5 subunits were determined in *blm3 $\Delta$*  cells compared with isogenic wild-type cells that express haemagglutinin (HA)-tagged  $\beta$ 5 instead of the endogenous protein (Fig. 2A). In parallel, *ump1 $\Delta$*  cells were analysed as a control for precursor stabilization. Immunoblot analysis showed that pro- $\beta$ 5 subunits are present in wild-type cells (Fig. 2A, lane 1), hardly detectable in *blm3 $\Delta$*  cells (Fig. 2A, lane 2) and accumulate in *ump1 $\Delta$*  cells (Fig. 2A, lane 3). One possible explanation for this finding is that the half-life of pro- $\beta$ 5 subunits is decreased in *blm3 $\Delta$*  cells compared with wild-type cells. Consequently, Blm3 would delay precursor processing.

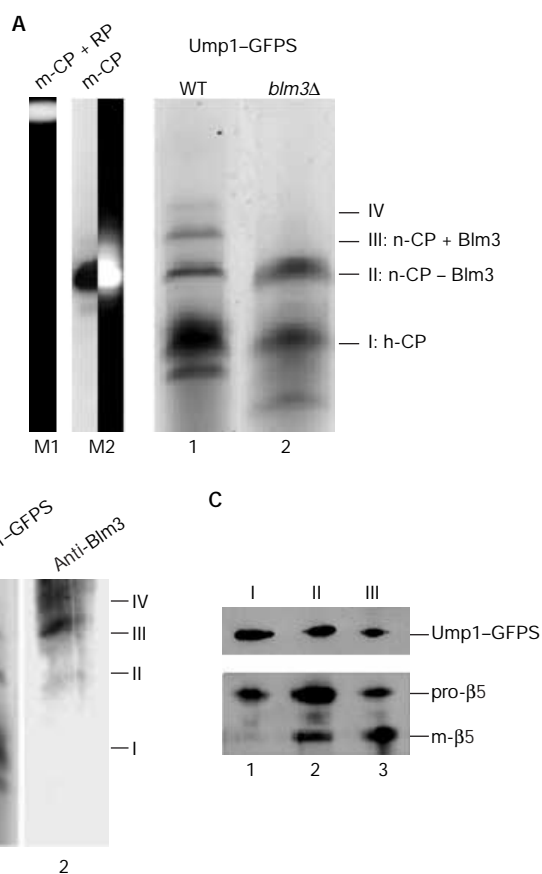
If this conclusion is correct, the kinetics of pro- $\beta$ 5 processing and the turnover of Ump1 should be accelerated in *blm3 $\Delta$*  cells compared with wild-type cells. To measure the kinetics of CP maturation, pulse-chase experiments were performed. A lack of Blm3 led to an increased processing rate of pro- $\beta$ 5 and an accelerated degradation of Ump1 (Fig. 2B), suggesting that Blm3 does delay the maturation process of the CP.

Is Blm3 an antagonist of Ump1? A *blm3 $\Delta$  ump1 $\Delta$*  double-knockout strain was created to test whether a lack of Blm3 can be compensated for by a lack of Ump1. However, *blm3 $\Delta$  ump1 $\Delta$*  cells showed an *ump1 $\Delta$*  phenotype, which is manifested in the stabilization of precursor complexes (data not shown).

Next, we investigated whether Blm3 is associated with early or late Ump1-associated precursor complexes, namely the h-CP or



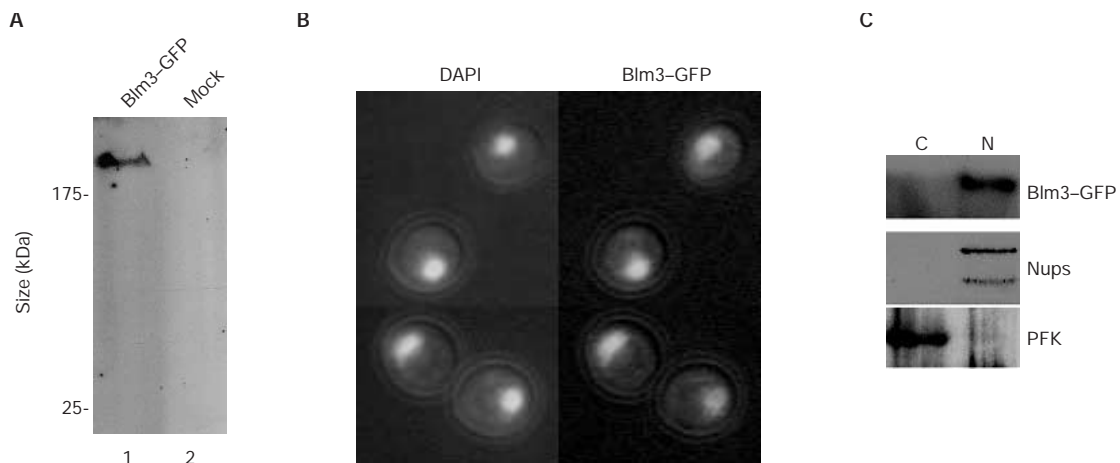
**Fig. 2** | Blm3 delays core particle maturation. (A) Wild-type (WT),  $\Delta blm3$  and  $\Delta ump1$  cells were grown to logarithmic phase (optical density of 1 at 600 nm), harvested, boiled immediately in sample buffer and subjected to SDS-polyacrylamide gel electrophoresis followed by immunoblotting. Proteins were probed for haemagglutinin (HA)-tagged  $\beta 5$  and  $\alpha 4$  subunits, the latter of which was used as a loading control. Unprocessed (pro-)  $\beta 5$ , matured (m-)  $\beta 5$  and  $\alpha 4$  are indicated. (B) Pulse-chase analysis, comparing the rates of pro- $\beta 5$  processing and Ump1 degradation in wild-type and  $blm3\Delta$  cells. Cells were pulse-labelled with  $^{35}\text{S}$ -Met/Cys for 5 min and chased for the lengths of time indicated. HA-tagged  $\beta 5$  and Ump1 were precipitated with anti-HA antibodies. Pro- $\beta 5$ , m- $\beta 5$  and Ump1 are indicated. Each pulse-chase analysis comparing  $blm3\Delta$  and wild-type cells was performed three times in parallel.



**Fig. 3** | Blm3 is associated with the nascent core particle but not with the half-assembled core particle. (A) Green-fluorescent-protein-streptactin (GFPS)-tagged precursor complexes, which were isolated by streptactin affinity chromatography from wild-type (WT; lane 1) and  $blm3\Delta$  (lane 2) cells expressing Ump1-GFPS instead of the endogenous protein, respectively, were separated by native polyacrylamide gel electrophoresis. The GFP-labelled precursor complexes were visualized by scanning the gel with a phosphorfluorimager. Bands were numbered (I–IV) and assigned to the half-assembled core particle (h-CP), or to the nascent CP (n-CP) with (+) or without (–) Blm3. Species I was assigned to the h-CP, as this species represents the main fraction of precursor complexes (Chen & Hochstrasser, 1996; Ramos *et al.*, 1998; Lehmann *et al.*, 2002). The n-CP was found to behave like the mature CP (m-CP) in native gels. Thus, the m-CP (Lehmann *et al.*, 2002) was run as a size marker (lane M2; the left half shows phosphorfluorimaging of the GFPS-tagged m-CP; the right half shows the chromogenic peptide-cleavage activity of the GFPS-tagged m-CP). As another control, the migration of the m-CP in association with the regulatory particle (RP) is shown (lane M1). (B) Native gels of Ump1-associated precursor complexes were scanned for Ump1-GFPS (lane 1), blotted, and probed for Blm3 (lane 2). Bands were assigned as above. Band IV probably represents the n-CP capped by two Blm3 molecules, but could not be characterized further due to limiting amounts of protein. (C) The wild-type precursor complex species (bands I–III) were excised from the native gel, subjected to SDS-PAGE, blotted, and probed for Ump1 and  $\beta 5$  (lanes 1–3 are derived from bands I–III, respectively). Ump1-GFPS, pro- $\beta 5$  and mature  $\beta 5$  (m- $\beta 5$ ) are indicated. Subunits of the RP were not detected in any of our preparations of Ump1-associated precursor complexes (data not shown).

the n-CP. For this purpose, we analysed precursor complexes from wild-type and  $blm3\Delta$  cells by native polyacrylamide gel electrophoresis (PAGE). As precursor complexes are not detectable by chromogenic peptide-cleavage products because they are proteolytically inactive, we used GFP-streptactin (GFPS)-tagged Ump1, which allowed the affinity purification of precursor complexes under native conditions (Lehmann *et al.*, 2002). GFPS-tagged precursor complexes from wild-type and  $blm3\Delta$  cells were run on native PAGE gels. Phosphorfluorimaging of the native gels identified the species of Ump1-associated precursor complexes

(Fig. 3A). Compared with the pattern in wild-type cells,  $blm3\Delta$  cells lacked the more slowly migrating species (Fig. 3A, compare bands III and IV in lanes 1 and 2). Immunoblotting analysis showed that the more slowly migrating species in wild-type cells are Blm3-associated precursor complexes (Fig. 3B). To analyse the protein composition, the species of Ump1-associated precursor complexes from wild-type cells (Fig. 3A, bands I–III) were excised from the native gel, subjected to SDS-PAGE, and blotted and probed for Ump1 and  $\beta 5$ . The predominant fraction of precursor complexes (Fig. 3A,B, band I) contained Ump1-GFPS and pro- $\beta 5$



**Fig. 4** | Blm3 is nuclear. **(A)** Western blot analysis of cells in which Blm3 is chromosomally replaced by a green fluorescent protein (GFP)-tagged version (lane 1) compared with mock-transfected (mock) cells (lane 2). Anti-GFP antibodies were used. **(B)** Direct fluorescence microscopy was used (Enenkel *et al.*, 1998) to monitor GFP-tagged Blm3 in living cells (left panel, DAPI (4',6-diamidino-2-phenylindole) staining of yeast nuclei, as visualized by the ultraviolet light filter superimposed with Nomarski optics; right panel, GFP filter superimposed with Nomarski optics). **(C)** Spheroplast lysates were fractionated into a cytosolic (C) and a nuclear (N) fraction, separated by SDS-polyacrylamide gel electrophoresis, blotted, and probed for Blm3, nuclear (Nups) and cytoplasmic (PFK) controls (Enenkel *et al.*, 1998).

(Fig. 3C, lane 1). The fractions of precursor complexes (Fig. 3A,B, bands II and III) that migrated slower than the predominant fraction contained Ump1-GFPs, pro- $\beta$ 5 and mature  $\beta$ 5 (m- $\beta$ 5; Fig. 3C, lanes 2 and 3).

As the h-CP contains only unprocessed  $\beta$ 5 subunits and represents the predominant fraction of precursor complexes in yeast (Chen & Hochstrasser, 1996; Ramos *et al.*, 1998), we assigned the precursor species I to the h-CP. Blm3 was not found in the fraction of the h-CP (Fig. 3B), which suggests that Blm3 has no impact on early stages of CP assembly. The ongoing processing of pro- $\beta$ 5 involves the short-lived n-CP. Thus, we assigned the precursor species II and III to the n-CP. As shown in Fig. 3B, Blm3 is part of the n-CP. These data show that Blm3 accompanies Ump1-associated precursor complexes only at a late stage of CP assembly.

We were now able to interpret our result that *blm3 $\Delta$  ump1 $\Delta$*  mutants have the same phenotype as *ump1 $\Delta$*  mutants. A deficiency of Ump1 disrupts the formation of precursor complexes that are already at an early stage of assembly, which results in the accumulation of h-CPs (Ramos *et al.*, 1998). As Blm3 follows Ump1 at a later stage of precursor assembly, a lack of Ump1 cannot be compensated for by a lack of Blm3.

As Blm3 plays a major role in CP maturation, Blm3 was expected to localize to the nucleus, similar to Ump1. To monitor the subcellular localization of Blm3 in living cells, *BLM3* was replaced in the chromosome by a GFP fusion construct using yeast genetics (Fig. 4A). Blm3-GFP behaved like endogenous Blm3 in density-gradient ultracentrifugation (see supplementary information online). Direct fluorescence microscopy (Fig. 4B) and biochemical fractionation of wild-type cells that expressed Blm3-GFP (Fig. 4C) showed that Blm3 localizes to the nucleus to the same extent as Ump1 (Lehmann *et al.*, 2002). The nuclear localization of Blm3 remained unchanged in *ump1 $\Delta$*  cells (see supplementary information online), which indicates that Blm3 does not require Ump1 for efficient nuclear import. GFP-labelled Ump1 and  $\alpha$ 4 subunits of the CP localized mainly to the nuclear periphery of *blm3 $\Delta$*  cells, as seen in wild-type cells (see supple-

mentary information online), which shows that Ump1-associated precursor complexes are not stabilized in the yeast nucleus due to the presence of Blm3.

## CONCLUSIONS

In this study, we describe Blm3 as a new component of nuclear CP assembly. Blm3 is part of the n-CP, a fraction of Ump1-associated precursor complexes. Our *in vivo* localization studies combined with biochemical fractionations show that Blm3 is associated with the n-CP in the nucleus. Thus, Blm3 is involved in the coordination of a late stage of nuclear CP maturation, and this complements our recently proposed model of nuclear CP assembly (Lehmann *et al.*, 2002).

As a lack of Blm3 results in an increased rate of precursor processing and accelerated degradation of Ump1, we assume that Blm3 functions as a checkpoint protein in preventing premature activation of the nuclear CP. Under stress conditions, this function of Blm3 may be particularly required in the biological context of DNA repair, which may explain why Blm3 was originally identified as a protein that protects cells from DNA damage (Evans Febres *et al.*, 2001).

With regard to PA200, the structural homologue of Blm3 in mammalian cells (Ustrell *et al.*, 2002), it remains to be determined whether Blm3 and PA200 are functionally related. PA200 has a stimulatory effect on the peptide-cleavage activity of the nuclear CP in mammalian cells. In yeast, our data provide no evidence that Blm3 functions as an activator of the mature CP. By contrast, we saw an inhibitory effect of Blm3 on proteasomal proteolysis *in vitro* and *in vivo* (see supplementary information online), consistent with our findings that Blm3 delays precursor processing as part of the n-CP. We assume that Blm3 joins the n-CP during the transition into the mature CP and that Blm3 interacts with the *de novo* synthesized m-CP.

As yeast cells that lack Blm3 are temperature sensitive and those that overproduce Blm3 are growth-inhibited, and because Blm3 controls a late stage of nuclear CP maturation, we propose that Blm3 is generally required for the regulation of nuclear CP maturation. In consequence, Blm3 may have an impact on nuclear CP activation.



**Table 1** | *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Reference
WCGa	<i>MATa1, his3-11,15 leu2-3,112 ura3 can GAL</i>	Enenkel et al., 1998
UCE1	<i>MATa1, UMP1-ProA::YIp5</i>	Derivative of WCGa
UCE2	<i>MATa1, UMP1-GFPS::YIp5</i>	Derivative of WCGa
UCE3	<i>MATa1, UMP1-HA::YIp5</i>	Derivative of WCGa
UCE4	<i>MATa1, ump1Δ::LEU2</i>	Derivative of WCGa; Ramos et al., 1998
UCE5	<i>MATa1, blm3Δ::HIS3, UMP1-GFPS::YIp5</i>	Derivative of BMF1
BMF1	<i>MATa1, blm3Δ::HIS3</i>	Derivative of WCGa
BMF2	<i>MATa1, blm3Δ::HIS3, ump1Δ::LEU2</i>	Derivative of UCE4
BMF3	<i>MATa1, blm3Δ::HIS3, UMP1-HA::YIp5</i>	Derivative of BMF1
BMF4	<i>MATa1, blm3Δ::HIS3, PRE2-HA::URA3 HIS3</i>	Derivative of BMF1
BBH1	<i>MATa1, PRE2-HA::URA3 HIS3</i>	Derivative of WCGa
BBH2	<i>MATa1, ump1Δ::LEU2, PRE2-HA::URA3 HIS3</i>	Derivative of UCE4
BAL1	<i>MATa1, Blm3-GFP-HA::URA3 HIS3</i>	Derivative of WCGa

METHODS

**Plasmids and strains.** Using homologous recombination techniques (Enenkel et al., 1998), endogenous *BLM3* was replaced by *BLM3-GFP-HA* by integrative transformation of a GFP-HA tag at the 3' end of *BLM3-YFLO06* using *SpeI/XhoI*-digested pBS-YFLO06-GFP-HA-URA3-HIS3. The *blm3Δ::HIS3* allele contains a deletion of 5,415 nucleotides starting from position -53 (numbered with reference to the ATG). *blm3Δ* cells and isogenic wild-type cells that express tagged versions of β5 and Ump1 instead of the endogenous proteins were generated as described in Lehmann et al. (2002). The chromosomal replacement of β5 (Pre2) by an HA-tagged version was achieved by using *Sall/XhoI*-digested pBS-Pre2HA-URA3-HIS3.

The strains used in this study are derived from the WCGa wild-type strain and are listed in Table 1.

**Protein chemistry.** Protein-A- and GFPS-tagged protein complexes were isolated by affinity chromatography using IgG sepharose and streptactin matrices, respectively (Lehmann et al., 2002). Fractionation of yeast-cell extracts into soluble and nuclear/ER fractions was performed as described (Lehmann et al., 2002).

Gel filtration on Superose 6 (Amersham) was performed in 50 mM Tris-HCl, pH 7.5, 2 mM ATP, 5 mM MgCl<sub>2</sub>, 1 mM dithiothreitol (DTT), 15% glycerol, as described in Ramos et al. (1998).

Proteasomal activity was measured in 20 mM Tris-HCl, pH 7.8, 5 mM MgCl<sub>2</sub>, 10 mM KCl, 1 mM DTT, for 30 min at 37 °C using 100 μM chromogenic substrates (Ustrell et al., 2002).

**Native gel electrophoresis.** Polyacrylamide gels (3.5–6.0%) were cast as described in Frentzel et al. (1994). Freshly prepared protein samples contained 10% glycerol and traces of xylene-cyanol blue. Gels were run at a cold temperature (8 °C) at 100 V for 30 min, and then at 200 V for ~2 h in 0.09 M Tris base, 0.09 M H<sub>3</sub>BO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, pH 8.1–8.4, 2 mM ATP, 2 mM DTT. GFP-tagged proteasomal complexes were visualized using Advanced Image Data Analysis (AIDA; Version 2.0; www.raytest.de) installed on a Phospho-Fluoro Imager FLA3000 (Fujifilm). For analysing the protein composition of the GFP-labelled complexes, the bands visualized by phosphorfluorimaging were excised from the native gel, boiled in sample buffer and subjected to SDS-PAGE.

**Other methods.** Anti-Blm3 peptide antibodies were raised in guinea pig against amino-terminal residues 2–18 of Blm3 (J. Pineda). Pulse-chase experiments were carried out as described in Ramos et al. (1998). Direct fluorescence microscopy was performed as described in Enenkel et al. (1998).

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank K. Janek for peptide-mass fingerprint analysis, R. Kraft for microsequence analysis, J. Dohmen, C. Mann and D. Wolf for strains and antibodies, B. Braun for pBS-Pre2HA-URA3-HIS3, B. Dahlmann for advice about proteasome enzymology and P.-M. Kloeetzel and W. Heinemeyer for discussions and critical reading of the manuscript. This work was supported by a Deutsche Forschungsgemeinschaft grant (EN 301/4-2) to C.E.

REFERENCES

Chen, P. & Hochstrasser, M. (1996) Autocatalytic subunit processing couples active site formation in the 20S proteasome to completion of assembly. *Cell*, **86**, 961–972.

Enenkel, C., Lehmann, A. & Kloeetzel, P.M. (1998) Subcellular distribution of proteasomes implicates a major location of protein degradation in the nuclear envelope-ER network in yeast. *EMBO J.*, **17**, 6144–6154.

Evans Febres, D., Pramanik, A., Caton, M., Doherty, K., McKoy, J., Garcia, E., Alejo, W. & Moore, C.W. (2001) The novel *BLM3* gene encodes a protein that protects against lethal effects of oxidative damage. *Cell. Mol. Biol.*, **47**, 1149–1162.

Frentzel, S., Pesold-Hurt, B., Seelig, A. & Kloeetzel, P.M. (1994). 20S proteasomes are assembled via distinct precursor complexes. Processing of LMP2 and LMP7 proproteins takes place in 13–16S preproteasome complexes. *J. Mol. Biol.*, **236**, 975–981.

Gavin, A.-C. et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature*, **415**, 141–147.

Glickman, M.H. & Maytal, V. (2002) Regulating the 26S proteasome. *Curr. Top. Microbiol. Immunol.*, **268**, 43–72.

Heinemeyer, W. (2000) in *Proteasomes: the World of Regulatory Proteolysis* (eds Hilt, W. & Wolf, D.H.), 48–70 (Eurekah.com/Landes Biosciences Publ. Co., Georgetown, Texas, USA).

Jäger, S., Groll, M., Huber, R., Wolf, D.H. & Heinemeyer, W. (1999) Proteasome β-type subunits: unequal roles of propeptides in core particle maturation and a hierarchy of active site function. *J. Mol. Biol.*, **291**, 997–1013.

Krüger, E., Kloeetzel, P.-M. & Enenkel, C. (2001) 20S proteasome biogenesis. *Biochimie*, **83**, 1–5.

Lehmann, A., Janek, K., Braun, B., Kloeetzel, P.-M. & Enenkel, C. (2002). 20S proteasomes are imported as precursor complexes into the nucleus of yeast. *J. Mol. Biol.*, **317**, 401–413.

Ramos, P.C., Hockendorff, J., Johnson, E.S., Varshavsky, A. & Dohmen, R.J. (1998) Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly. *Cell*, **92**, 489–499.

Russell, S.J., Steger, K.A. & Johnston, S.A. (1999) Subcellular localization, stoichiometry, and protein levels of 26S proteasome subunits in yeast. *J. Biol. Chem.*, **274**, 21943–21952.

Ustrell, V., Hoffman, L., Pratt, G. & Rechsteiner, M. (2002) PA200, a nuclear proteasome activator involved in DNA repair. *EMBO J.*, **21**, 3516–3525.

Wilkinson, C.R., Wallace, M., Morpew, M., Perry, P., Allshire, R., Javerzat, J.P., McIntosh, J.R. & Gordon, C. (1998). Localization of the 26S proteasome during mitosis and meiosis in fission yeast. *EMBO J.*, **17**, 6465–6476.